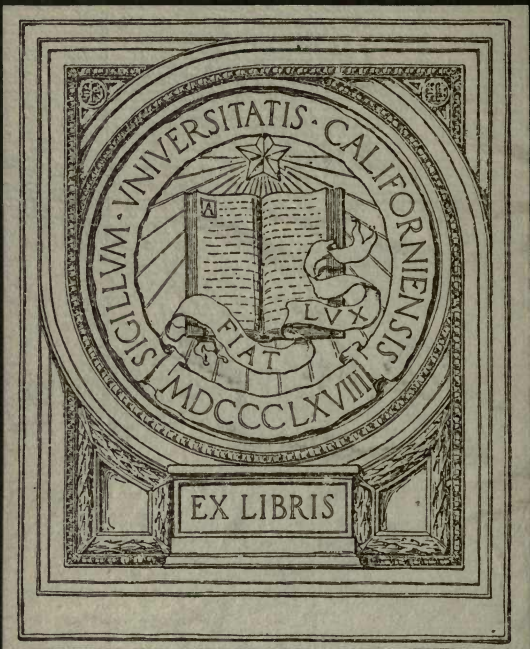

# HOW TO USE AND CARE FOR THE

# MICROSCOPE

COMPLIMENTS OF

SPENCER LENS CO.

BUFFALO, N. Y.

# How to Use and Care for

# THE

# MICROSCOPE

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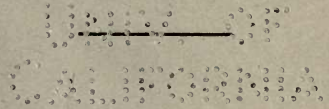
A Simple Treatise on the Use of the Microscope
especially adapted to laboratory work.



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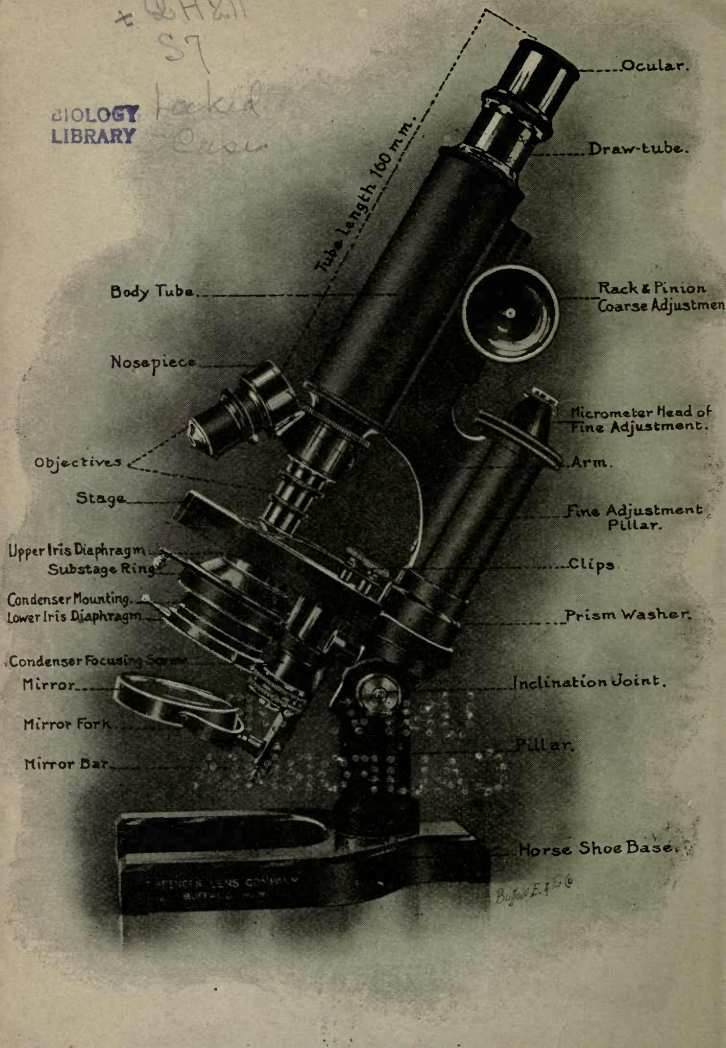

# PREFACE

A microscope,—like any other instrument of precision,—cannot be made to do its best work without some knowledge on the part of the user as to how to keep it in perfect condition, and an intelligent understanding of its parts, their use and relations.

The purpose of this little book is to bring before you some practical helps in clear concise form, which will enable you to obtain the best possible results when working with the microscope.

We make no attempt to go into the optical principles involved in the microscope except in so far as it is necessary to direct in its use. For these we refer you to more pretentious works,—The Microscope, by Dr. S. H. Gage; The Microscope and its Revelations, by Carpenter & Dallinger.

Let it be remembered that no amount of direction will take the place of good judgment and careful painstaking effort on your part, and that it is only the perfect adjustment of every part in relation to every other part which brings the best results. The neglect of one detail may destroy the virtues of all the others.

It is with a desire to help and a hope that your work with the microscope may prove pleasant and profitable that we present this little volume.

***SPENCER LENS CO.***

## DONT'S

*Don't* allow dust and dirt to settle on the microscope.

*Don't* carry the microscope by the arm.

*Don't* use alcohol on the microscope.

*Don't* expect too great a range in the fine adjustment.

*Don't* take the fine adjustment apart.

*Don t* bring the objective into contact with the cover glass.

*Don't* fail to focus up before turning the nosepiece unless you *know* the objectives are parfocal.

*Don't* forget that high powers have short working distances.

*Don't* focus down with the eye at the eyepiece.

*Don't* fail to secure good, even illumination.

*Don't* drop the objectives and oculars.

*Don't* try to take an objective apart.

*Don't* try to work with dirty lenses.

*Don't* try to clean them with a dirty cloth.

*Don't* fail to clean oil from an immersion lens immediately after using.

*Don't* try to work with an immersion lens when there are air bubbles in the oil.

*Don't* use high powers when low ones will do.

*Don't* use higher oculars than necessary.

*Don't* expect a lens to work at its best unless used on a cover thickness, and with a tube length, for which it is corrected.

*Don't* shut one eye.

*Don't* get discouraged if desired results do not come immediately.

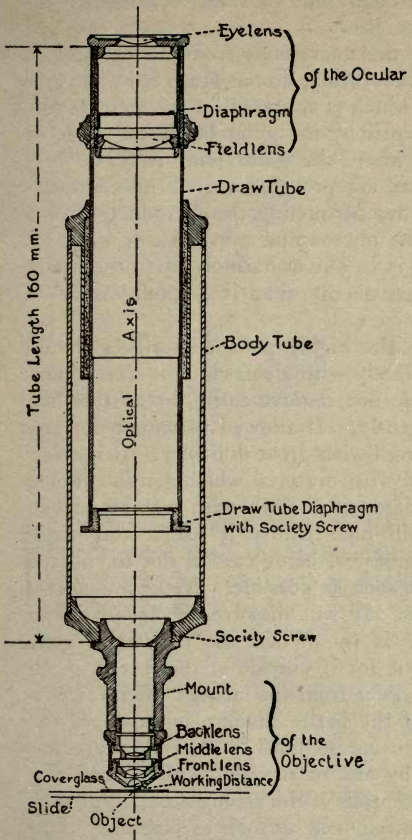


FIG. 1. ALL OF THE PARTS MENTIONED IN THE TEXT ARE INDICATED ON THIS FIGURE AND THE FRONTISPIECE.

## PART I

# CARE OF THE MICROSCOPE

### THE STAND

Every microscope regularly leaves the factory in a case made especially for it in which it fits securely. If it is to be carried any distance it should be carried in the case. It should be left in the case when not in use unless some other means are provided to protect it from dust.

Dust settling upon the highly polished surfaces is apt to scratch them when it is removed. It works into the bearings of the instrument, making them work hard and unnecessarily wearing them.

The microscope ought not to stand continually exposed to direct sunlight.

When removing the microscope from the case, or in handling it in the laboratory, do not grasp it by the arm (see frontispiece), which is actuated by the delicate fine adjustment. Grasp it by the pillar below the stage or by the stage just where the pillar joins it. The best modern instruments are provided with a new fine adjustment at the side, permitting the introduction of a handle by which the microscope can be easily handled. All rough handling is so out of harmony with the use of such a delicate instrument that it is unnecessary to caution against it.

Finger marks on the highly polished surfaces should be removed immediately with a soft cloth or *clean* chamois skin. If they do not remove easily, breathe on the surfaces and rub gently. If allowed to remain for any time they are removed with great difficulty. If the surfaces become soiled with material which gentle rubbing will not remove, dampen a cloth with water and rub gently. If this will not remove it use a very little xylol, ether or chloroform, being careful not to rub too hard, and to dry as soon as possible. *Never use alcohol* on lacquered parts. It will dissolve the lacquer,—no matter how dilute it is. When the lacquer is gone nothing can be done for it outside of the factory. All reagents should be kept from the lacquer wherever possible. On most of the better modern microscopes the more exposed parts are so finished that they are not seriously affected by any of the above mentioned reagents, with the exception of the strong acids. All such reagents should be removed as soon as possible. This finish is generally a black enamel or a gray or black plating. Sometimes this finish is extended to the upper parts of the stand.

***Stage.*** On account of its exposed position the finish of the stage is worthy of special mention. The stages of the cheaper microscopes are finished with a preparation which gives the brass a dull black appearance. They are easily cleaned as directed above. When they become gray and dingy a very little of one of the heavier oils rubbed upon them will often make them black again, unless the finish be worn off. The stages of all the better microscopes are covered with hard rubber which is not permanently effected by any of the ordinary laboratory reagents. Should the stage become soiled with balsam, immersion oil, or anything which water will not remove, it can be cleaned with xylol or chloroform. The xylol will turn the black stage to a dull gray, but a little of some heavy oil rubbed upon it will restore the original black. If the gray color is of long standing it may be necessary to leave the oil on some time. Wipe off the oil thoroughly when it has done its work.

***Inclination Joint.*** All of the better microscopes are provided with the inclination joint by which the body can be inclined to any angle between perpendicular and horizontal. Once in a while this joint wears loose so that the microscope will not remain at the desired angle. This can be tightened by tightening the nuts on the ends of the inclination axis with a heavy screw driver if the nut is slotted, or with a "spanner" if the nut it provided with two small holes. A pair of round nosed pliers will serve the purpose nicely in the absence of the spanner. With any of the tools great care should be taken not to mar the nuts. On most of the modern instruments the pin which forms the axis is slightly conical and the necessary friction is obtained by drawing the cone tighter into its bearings. This necessitates the

secure the pin and put it in place. This ought to be done by the maker or an experienced mechanic.

In some cases, especially in microscopes (continental type) where the prism is used in the fine adjustment the lubricant in the prism becomes gummed so that the adjustment fails to respond promptly, and then jumps. The bearings should be thoroughly cleaned and oiled with paraffin oil or watch oil. This ought to be done by the manufacturers because the mechanism is so delicate that even though safely taken apart, it would be put together and adjusted with great difficulty.

***Draw Tube.*** The draw tube should work easily and smoothly. With those which are nickeled and sliding in a cloth lined sleeve, little trouble will be found. Where they are not nickeled, care must be given them similar to that described for the sliding tube coarse adjustment. In pushing in the draw tube be careful not to push down the body tube and thereby run the front of the objective into the cover glass.

***Substage.*** The rules given above apply to the parts of the substage in general. The threads on the quick acting screw on the instruments so provided are apt to become gummed, making it hard to focus the condenser. This gum is easily "cut" with xylol or chloroform, permitting the screw to work easily.

If the leaves of the iris diaphragms become rusted or gummed, clean them with xylol, and oil them thoroughly by opening and closing the diaphragm several times to evenly distribute the oil over the leaves. Should the leaves become bent or misplaced, submit them to the maker or a skilled workman.

***Nosepiece.*** When bought at the same time the nosepieces and objectives of all the best makers are now

so made that the objectives are *parfocal: i. e.*, when one lens is in focus the others on the nosepiece will be in fairly good focus when they are swung into the optical axis. They are also approximately centered so that a point in the center of the field of one lens will be in the field of the others. To accomplish this, each set of objectives must be especially fitted to their particular nosepiece. Care should then be taken in the laboratory not to interchange objectives. Be careful not to bend the nosepiece in any way so that the objectives will be thrown out of center. Unless you are *positive* that your lenses are parfocal, always focus up slightly before turning from a lower to a higher power; otherwise you are apt to swing the front of your objective against the cover glass and injure both the specimen and objective. Remember that objectives made parfocal for one tube length are not parfocal for a different length. If occasion occurs for screwing the objectives on or off from the nosepiece, always use both hands, never letting go of the objective entirely, so as to preclude any liability of injuring them by dropping.

### *THE OPTICAL PARTS*

With the stand cleanliness is a virtue; with the objectives it is also an absolute necessity. If the lenses are dirty they should be wiped gently with Japanese lens paper, which can be obtained from any dealer in microscopical supplies. It is so cheap that one can hardly afford to use anything else on his lenses,—especially the objectives. If the lens paper is not obtainable a soft old linen handkerchief is best, *providing it is clean.* Avoid chamois skin. The natural oils in it soil the surface of the lens, and its aptitude to catch and hold dirt makes it unsafe.

Never rub a lens hard with anything. Avoid touching

the surface of a lens with the bare hands. The perspiration is hard to remove.

***Objectives.*** If the front lens of an objective becomes soiled so that gently wiping will not clean it, breathe upon it and then wipe gently with lens paper or some soft linen. If this does not remove the soil, moisten the paper with xylol or chloroform, being careful not to use too much. Although the necessity of using these reagents is unfortunate, it is better to use them and wipe the lens gently than to apply too much friction.

An immersion objective should always be cleaned immediately after using. It can then be cleaned by gently wiping with a piece of lens paper. If the oil is allowed to dry, xylol or chloroform must be used to clean the lens. The oil collects dust and grit, which are apt to scratch the lens.

If any dust settles on the back lens of the objective it is best removed by a camel's hair brush. An eyepiece should always be left in the tube to keep dust from settling into the objective on the lower end.

*Never attempt to take an objective apart.* If it has any ailment serious enough for this it is serious enough to go to the maker.

***Oculars.*** The oculars should be wiped as directed for the objectives. Sometimes a grayish film forms on the inner surfaces of the lenses. This necessitates removing the lenses from the tube and wiping their surfaces.

***Condenser.*** What has been said of the eyepiece applies to the condenser. The inner surfaces of the lenses of the condenser should be cleaned if the condenser is not clear after cleaning the outer surfaces. The objective cannot do its best work unless the condenser is clean.

***Mirror.*** The surfaces of the mirror demand the same care and treatment as the lenses.

## PART II.

# THE USE OF THE MICROSCOPE

### POSITION

In choosing a place to work one should select a comfortable position where he can obtain the best light available and have room for his microscope and necessary accessories and reagents. There is some controversy in regard to the using of the inclination joint. There is no harm in using it if it is more comfortable to do so. If one is working with fresh mounts or fluids, the horizontal stage is necessary. Because such preparations are so often used in the laboratory, it is best for one to train himself to use the microscope with the tube in the perpendicular position and make it a rule to keep it in that position.

Make it a rule to work with both eyes open, and if possible, use either eye interchangeably. A very little practice will enable one to do so. By paying attention to this and proper lighting, there is no reason why any reasonable amount of work with the microscope should injure the eyes.

### LIGHT

The best light is obtained from white clouds, although some authorities claim that the light from the blue sky is best. Avoid the use of direct sunlight. If the room is so situated that the sun shines in, use white shades to modify the sunlight. If possible, select a window which is free from cross bars, wire nettings, etc., and which is some distance from swaying branches of trees.

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For long continued work on any one subject artificial light has one advantage over daylight in that it is constant in quality and intensity. The best artificial light is a Welsbach burner. A whitened incandescent bulb is good. Ordinary lamplight can be used very successfully. In using artificial light it is best to use a bull's eye condenser between it and the mirror. It is also best, wherever possible, to use a blue glass between the light source and the specimen. Some workers make a glass globe filled with ammonia copper sulphate serve the purpose of both the condenser and the blue glass. It is so mounted in a shade as to exclude all other light from the microscope. An eye shade, or some shade cutting off all light from the microscope excepting that which strikes the mirror, is often desirable.

### *FOCUSING*

After seeing that an objective (low power) and an ocular are in place put a transparent or semi-transparent specimen on the stage, swing the mirror bar to the median line, take hold of the edge of the mirror and adjust it so as to illuminate the object as evenly as may be judged by looking directly at it.

Focus the body tube down by means of the coarse adjustment until the objective nearly touches the cover glass, being careful not to touch it. Then with the eye at the eyepiece, focus up carefully with the coarse adjustment until the specimen comes plainly into view. Be careful not to pass by this focal point without noticing it. This is likely to occur if the light be too intense and the specimen thin and transparent. If the sliding tube coarse adjustment is used, focus carefully by giving the tube a spiral movement.

When the object is brought fairly well into focus by means of the coarse adjustment use the fine adjustment

to obtain the sharpest focus to bring out details. Do not expect too great a range in the fine adjustment. It is even more dangerous to focus down to any extent with the fine adjustment than with the coarse adjustment, because any impact of the front of the objective on the cover cannot be as easily felt. While moving the specimen about to observe different parts of it, it will be necessary to continually work the fine adjustment to keep the object in focus. It is always well to move the specimen when trying to get a focus, for without the movement one may be trying to focus upon a point where there is no object, and again, the moving object is more apt to be noticed as the lens comes into focus.

It will be noticed during this movement that the microscope reverses the image, and that the specimen seems to move in the direction opposite to that in which it is moved. This, along with the fact that the microscope magnifies the movement as well as the specimen, is perplexing at first and makes it difficult to move the specimen just where it is wanted, and no farther. With practice comes the delicacy of movement which enables one to put the specimen just where he wants it.

The beginner should always use the low power objectives and oculars first. The low power objectives have longer working distances and are not so apt to be injured. They always show a larger portion of the specimen and thus give one a better idea of the general contour. After obtaining this general idea the higher powers can be used to bring out greater detail in any particular part. If the objectives are par-focalized and centered on a nosepiece as described on page 11, the change of objectives is made by simply turning one objective out of the optical axis and the other into it without the necessity of re-focusing (except for a slight turn of the fine adjustment) and again hunting up the

particular spot desired, for if this spot is in the center of the field of the low power it will be somewhere in the field of the higher power. It is too much to ask of the maker that the lenses be made absolutely parfocal and centered. The delicacy of the centering can be appreciated when the magnification and the extremely small portion examined is considered. When the objectives are not thus fitted to the nosepiece, re-focusing and again hunting up the object are necessary. In doing so we repeat the caution to always focus up before turning the nosepiece. When no revolving nosepiece is used the change of objectives means the unscrewing of one and the screwing of the other into its place, and re-focusing as before.

### *ILLUMINATION—WITHOUT SUBSTAGE CONDENSER.*

***Central Light.*** It has been necessary in the foregoing paragraphs to secure some light upon the specimen, but no directions have been given as to the proper illumination of the same. Accuracy of results depends upon correct illumination more than any other one thing. A vast majority of all microscopic work is done by light transmitted through transparent or semi-transparent objects. We will at present consider only such objects. The matter of illuminating opaque objects will be taken up later. The mirror is placed below the stage as a convenient means of reflecting the light through the object into the objective. It is plane on one side and concave on the other. The concave mirror is always used when the substage condenser is not used, except in the case of very low power objectives, when it is best to use the plane mirror.

When the light is thrown upon the specimen and the objective focused as previously directed, remove the ocular and look into the tube at the back lens of the

objective. With the medium and higher power (16mm and above) objectives the minimized image of the mirror with its mounting will be seen. Swing the mirror bar to the median line and as nearly as possible arrange the mirror so that its mounting will be concentric with the periphery of the back lens of the objective. All of the better microscopes are made with a "center stop," indicating when the mirror bar is in a line parallel with the optical axis of the microscope. This is done because central, or axial, light gives a symmetrical illumination, which is best for observing the large proportion of transparent objects. This in itself does not insure axial light. The mirror must be so turned that the rays of light, or the axis of the cone of light, reflected from it enter the objective parallel to its axis. This cannot always be done. Other considerations are more important than exact central light. In working with daylight, reflections from trees, window sash, etc., are apt to be seen on the mirror. If the whole microscope cannot be so shifted as to clear the mirror of these reflections the mirror itself should be turned so that, if possible, no images will appear upon it.

If artificial light is used the mirror should be so turned that the image of the light is seen in the center of it. The more nearly this image covers the mirror, the better. If a bull's eye condenser is at hand, so place it between the light source and the object that a sharp image of the light source will be seen in the center of the back lens of the objective.

If the above rules are followed it will be found upon replacing the eyepiece that the field is evenly illuminated. It may be necessary to vary the width of the cone and the quantity of light by use of the diaphragm which is always placed on all the better microscopes as nearly as possible even with the top surface of the stage. "When no condenser is used the size of the opening in

the diaphragm should be about that of the front lens of the objective. For some objects and some objectives this rule may be quite widely departed from; one must learn by trial."* The concave mirror acts as a lens and has a focus like a lens. It will often be found that by carefully focusing the mirror, details will be brought out clearly which otherwise would be but dimly seen.

It may be found that in focusing up and down, the image shifts slightly from right to left, or to and fro. This may possibly be due to an imperfection in the microscope, but if the instrument is in good repair, and from any one of the reputable makers, the chances are more than likely that the shifting is due to oblique light, even though the mirror bar may be in the median line. This is even more apparent with a condenser than without it. Manufacturers are often condemned because of a mistaken idea that the mirror bar in this position means axial light. A slight turn of the mirror will stop the shifting and give axial illumination. When there is no lateral motion in focusing, the light is centered.

***Oblique Light.*** Some objects, such as diatoms, rulings, etc., are better defined when oblique light is used. This is accomplished without the condenser by swinging the mirror out of the optical axis and so turning it as to throw as much light as possible upon the object. When the ocular is removed the image of the mirror will be seen at one side o the center of the back lens of the objective. When focusing a decided lateral motion of the object will be noticed.

### *ILLUMINATION—WITH SUBSTAGE CONDENSER*

***Central Light.*** All of the better microscopes are provided with a condenser fitted beneath the stage, which brings parallel rays of light to a focus at a point above

*The Microscope.—Gage.

its upper surface. With the lowest powers a condenser is not needed, but for the medium and higher powers the condenser not only furnishes the amount of light needed, but provides an easy means of providing each objective with a cone of light suitable to its aperture.

Condensers are of two great classes—the archomatic and non-achromatic. The achromatic are much the better, and are indispensable for photo-micrographic work, but as the non-achromatic (Abbe) is in such general use on account of its lesser cost and because it is sufficient for ordinary work, we will consider it especially. The same general rules apply to both, which are fundamentally the same as those given before, with the exception that in most cases the plane mirror is used because, as stated above, the condenser is made for parallel rays of light.

After removing the ocular, turn the mirror so that the back lens of the objective is fully and evenly illuminated and, if possible, free from any images of trees, window sash, etc. If these images cannot be dispelled by turning the mirror, use the concave mirror. Slightly lowering the condenser will also accomplish the end. There is an objection to both of these methods, which will be explained later.

When this is accomplished the proper cone of light must be secured by opening or closing the diaphragm below the condenser.

A good general rule is to close the diaphragm so that in looking at the back lens of the objective the diaphragm opening, which can be plainly seen, appears to be about half the diameter of the back lens of the objective when it is in focus. Then with the ocular in place change the opening to give the best results. The thinner the tissues and the greater the contrasts the larger the cone of light which may be used. Thicker tissues and those with less

contrast require a narrower cone, gaining thereby greater depth of sharpness (penetration). The narrower the cone the flatter the field appears. Very few objects permit of a cone which fills the back lens of the objective and in no case should the diameter of the iris diaphragm appear to be larger than the diameter of the back lens when the ocular is removed.

When objectives of over 1.0 N. A. (see p. 25) (immersion objectives) are used, the full aperture of the condenser cannot be utilized without immersing it, i. e., placing a drop of oil between it and the lower surface of the slide. This is seldom practiced in general laboratory work on account of its inconvenience, but it is necessary to the most critical work.

In working with artificial light it is always best to use a bull's eye condenser. If no bull's eye is available, use the concave mirror and so turn it that the image of the light source appears in the centre of the back lens of the objective when the ocular is removed. When the bull's eye is used, select the plane mirror and so place the light source and bull's eye that the image of the light source appears natural size on a cardboard placed at the back of the condenser. Remove the bull's eye and put the light source in its place. Focus the substage condenser so that the image of the light source appears in the plane of the object. This is best seen by using a low power objective and ocular. Now put the bull's eye in its former position after removing the light to its original place, or as before, so arrange the light source that a sharp image of the light source appears on the back of the condenser iris, or a card placed against it. This will give an even illumination. A blue glass should also be used beneath the condenser, unless the blue globe mentioned on page 14 is used. This modifies the yellow artificial light.

***Oblique Light.*** Even though both the condenser and iris are centered, central or axial light is not obtained, unless the rays of light, or the axis of the cone of light, from the mirror enter the condenser parallel with its axis. This fact is often forgotten as was pointed out before.

Beside the turning of the mirror oblique light can be obtained in a greater degree by decentering the lower iris and in the best microscopes the obliquity can be obtained from any azimuth by revolving the diaphragm mounting.

With the simple diaphragm mounting with a ring beneath the diaphragm for blue glass oblique light can be obtained by slipping a card between the ring and diaphragm in such a manner as to let the light into one side of the condenser only.

### *ILLUMINATION—OPAQUE*

There are some objects which cannot be made transparent and must be examined by reflected light. When low powers are used and the mirror brought above the stage the concave mirror is sometimes sufficient. The bull's eye condenser gives better results. In using it some care must be exercised to light the object and at the same time cast as few shadows as possible.

Where high powers with consequent short working distances are necessary, light must be thrown down through the objective and reflected back again to the eye by a prism placed in the objective mount or above it. Artificial light is necessary with such an arrangement.

### *FOCUSING THE CONDENSER*

Nearly every substage is provided with a means for focusing the condenser. The condenser does its best work only when the rays of light passing through it are focused sharply on the object. If there are any

reflections from the mirror of trees, window sash, etc., they will show on the back lens of the objective when it is focused and when the condenser is in this position. The operator must decide for himself as to whether he gets better results with the sharp focus and the images, or without either. If he decides to retain the images he can get best results by turning the mirror so that they are as symmetrically distributed over the back lens as possible. This emphasizes the importance of a clear, open source of light.

### *CENTERING THE CONDENSER*

For central light the axis of the condenser should coincide with the axis of the objective and the center of the opening of the diaphragm beneath the condenser should also be in this axis. On most of the medium grade microscopes the iris is so fastened to the condenser that it is concentric with its axis, and both are generally centered with the optical axis of the body tube before leaving the factory. This can be tested by a simple means. Close the diaphragm to its smallest aperture and notice this aperture through the back lens of the objective. If the condenser is centered this opening will appear in the center of the lens and will remain concentric with the periphery of the lens when the objective is focused up and down. The periphery of the top surface of the condenser will also be concentric with the periphery of the back lense of the objective.

On the more expensive microscopes there are little screws provided for moving the condenser in and out of center. By means of these the condenser can be centered by observing the above rule. On these instruments the iris diaphragm is brought in and out of center by a rack and pinion, a click indicating when it is centered.

All objectives are corrected to a certain tube length (160mm by most makers—Leitz 170mm) and all objectives in fixed mounts of over .70 N. A. are corrected to a definite thickness of cover glass as well. (Zeiss .15mm .20mm; Leitz, .17mm; Bausch & Lomb and Spencer, .18mm). These objectives give their best results only when used with the cover glass and tube length for which they are corrected. As indicated in the frontispiece, the tube length extends from the eye lens of the eyepiece to the end of the tube into which the objective or nose piece is screwed. If a nosepiece is used the draw tube must be correspondingly shortened. If the cover glass is thinner than that for which the objective is corrected the tube must be lengthened to obtain best results; if thicker, shortened. The more expensive objectives are provided with adjustable mounts by which the distances between the lens systems may be changed to compensate for difference of thickness of cover. They are successfully used only in the hands of an expert. One of them out of adjustment is worse than an ordinary objective.

### EQUIPMENT

A microscope equipment may be very elaborate and some work requires such an outfit, but every microscope to be efficient should be provided with at least two objectives, and preferably two oculars. If but one ocular is bought the 6x (No. 2, or 1½″) is best. If two are provided, the 4x and 8x (No. 1 and 3, or 2″ and 1″) are preferable. For ordinary biological, histological and pathological work the most desirable and most universally used objectives are the 16mm (⅔″) and 4mm (⅙″). The equivalents of these in European nomenclature are Nos. 3 and 6, or A and D, according to the maker. In some cases where a higher power is desired a 3mm (⅛″ or No. 7)

is used. For counting blood corpuscles a 5mm ($\frac{1}{5}''$) or a narrow angled 4mm ($\frac{1}{6}''$) with a long working distance for working through the thick cover of the blood counter is used. For entomological work and other low power work a 40mm ($1\frac{3}{4}''$) is very desirable. If bacteriological work or special cytological work is to be done, an immersion objective is indispensable. The 2mm ($\frac{1}{12}''$) is most used. For extreme work the 1.5mm ($\frac{1}{16}''$) is called into use. An immersion objective is one which is so corrected that a drop of oil (water, if it is a water immersion) must be used between the front lens and the cover glass. They are used like any other objective excepting for the placing of this drop of oil and for the fact that their shorter working distance requires greater care in handling. It is best to place the drop of oil upon the front lens of the objective with the little wire or rod which accompanies every immersion oil bottle. Any bubbles or dirt in the oil can be more easily detected in this way. It is a little unhandy to do this where the objective is screwed on the nosepiece. Many workers prefer to put the drop on the cover below the objective. Great care should be taken to exclude dirt or air bubbles. Dirt particles are apt to scratch the lens, and bubbles set up refractions which greatly interfere with the well working of the lens. If the bubbles are present they can be easily seen by removing the ocular and looking down into the objective. They must be removed even though it be necessary to wipe off all the oil and start over. The bubbles are very apt to occur if the oil contact is broken several times in an attempt to focus. If the full aperture of the objective (or any aperture greater than N. A. 1.0) is to be used the condenser must also be immersed as described on page 20.

Where the very best possible resu.ts are demanded the apochromatic objectives with compensating oculars are

used. They are made in the same powers as indicated above and are superior to the achromatic lenses because they have a more perfect chromatic and spherical correction.

### *OPTICAL QUALITIES OF OBJECTIVES*

***Numerical Aperture.*** N. A.=n. sin u. This term was introduced by Abbe. n. is the refractive index of the medium between the object and the front lens of the objective (air in case of dry objectives and water or oil in case of immerson objectives), and u is half the angular aperture.

Several important qualities of the objective depend upon the numerical aperture.

(*a*) Resolving Power. This is directly proportional to the numerical aperture and represents the ability of the objective to show detail in the image of the object. The higher the numerical aperture, the greater the resolving power, and the finer the detail we may expect to see in the image.

(*b*) Depth of Sharpness, or Penetration. This is the power of an objective to show sharply objects lying in different planes, one above another, without the necessity of focusing up and down. The depth of sharpness is in inverse ratio to the numerical aperture. Therefore the lens of low numerical aperture has little resolving power and great penetration. The lens of high numerical aperture has great resolving power and little penetration,—unless it be used with a narrow cone of light which practically makes it a lens of low aperture with the qualities of such a lens.

(*c*) Illuminating Power. The brilliancy of the objective increases with the square of the numerical aperture of the objective. An objective of .40 N. A.

will give an image four times as brilliant as one of .20 N. A., provided the magnification is the same and the full cone of the illumination is used in both cases.

***Magnifying Power.*** The magnifying power of an objective is in inverse ratio to its focal distance. An objective of 2mm focal distance will give, with the same ocular, a magnification eight times greater than one of 16mm focal distance. Numerical aperture and magnifying power are of little advantage if the definition is not good.

***Definition.*** The definition of an objective is characterized by the cleanness and sharpness of the outlines of the image.

Definition depends upon the corrections for chromatic and spherical aberrations, and the workmanship;—the centering of the lenses, etc.

CHROMATIC ABERRATION is due to the fact that a ray of white light passing from one medium to another of different refractive index at any angle other than 90° to the surface between them is refracted and dispersed into its component colors.

SPHERICAL ABERRATION is due to the fact that a spherical surface cannot bring a beam of light which passes through its vertex to the same focus as that of a beam of light passing through any other zone.

Both aberrations are corrected by the use of different kinds of glass (crown and flint) combined as double and triple lenses in the objective. Neither can be corrected absolutely for all colors in an achromatic objective. Apochromatic objectives approach the ideally corrected objective almost to perfection.

An objective can be tested for chromatic correction by using a narrow cone of oblique light and a coarse

grating. Abbe's test plate is best. Diatoms are good. No stained object should be used.

If the spherical correction is perfect (see next paragraph) and one side of a line passing through the center of the field shows a clear, narrow, greenish yellow border, while the other side is fringed with a violet red (seconddary colors) the objective is chromatically corrected. The colors shown in the higher power objectives are of a more primary character, *i. e.*, nearer the yellow and blue. Apochromatic objectives show no color borders in this test.

The spherical correction of an objective is perfected for a certain thickness of cover glass and a certain tube length, and is influenced greatly by any variation in either. This is especially true with the high power dry objectives. The homogeneous immersion objectives are not sensitive to the variation in the cover thickness because the immersion oil between the cover glass and the lens is of the same refractive index as the glass. They must be used however, with the proper tube length. In testing an objective for its spherical correction it is therefore very important to supply the proper thickness of cover and tube length. It is manifestly unfair to judge an objective on this point without complying with these conditions. The test for spherical correction can be made on the same object as used for the chromatic test. If the edges of the lines in the center of the field appear equally sharp and clear when illuminated by either a narrow central cone of light or a narrow oblique cone without having to change the fine adjustment the objective is spherically corrected. The color remnants mentioned above will be clear and transparent, while, if the lens is poorly corrected spherically, these borders will appear muddy and turbid. Defects in spherical corrections can often be corrected by using cover glasses suitable to them, also by changing the tube

length. The fact that the periphery of the field is not in focus at the same time as the center does not bespeak a lack of spherical correction, but a lack of *flatness of field* with which it is often confounded.

***Flatness of Field*** depends not only upon the objective itself, but upon the ocular and the cone of light used, whereas the spherical aberration is inherent in the objective itself. No field is absolutely flat. It is a desirable quality in a lens but spherical and chromatic corrections should never be sacrificed for it. Some lenses appear to be "flatter" than they really are, because their corrections are so poor that little contrast is noticed between objects in the center of the field and at the edge. Narrow cones of light give a flatter field than wide ones. Thin objects are more critical tests for flatness of field than thick ones.

***Working Distance*** is the free distance between the cover glass and the objective when the latter is focused. It decreases generally with increasing power and numerical aperture of the objective. Of two lenses with the same focal distance the one with the higher N. A. will have the shorter working distance. The working distance also depends on the mounting of the front lens. If the lens has a prominent mounting projecting beyond its surface the working distance is lessened thereby.

### *THE OCULARS*

A certain magnification by the ocular will be necessary, and sufficient, to bring out all the detail in the image which can be secured from the numerical aperture of the objective. If we use a higher ocular we lose depth of sharpness and size of field, since they are both inversely proportional to the magnification. We also lose illumination, which varies inversely as the square of the magnification.

We therefore get the greatest effectiveness out of an objective,—the largest field, the greatest penetration, and the best illumination,—by using the lowest magnification which makes all the detail in the image visible. If we increase the magnification beyond this point we do so at the expense of other good qualities.

Lengthening the tube increases the magnification proportionately.

### *FINAL HINTS*

Sometimes the worker may have faithfully carried out all the directions heretofore given and been assured that his lenses possess the above named qualities as they ought, yet be unable to obtain the desired results. He may be working with a water mount and his dry objective become "immersed" in some water which has worked to the top of the cover glass. His objective may be dirty from a previous "immersion," or it may have some other dirt upon the front lens. The field may be covered with specks which revolve when the ocular is turned. The field may be dim or hazy, due to dirt on the back of the objective or a film on the inner surfaces of the lenses of the ocular, or because of moisture settling on the lenses because they have just been brought from a cold into a warm room. He may see great streaks on his field, which are due to his own eye lashes, or he may see small, slowly moving bodies floating across the field. With the exception of this last, the ailment has only to be mentioned to suggest the remedy. The muscae volitantes, as these last named bodies are called, are little specks or shreds in the vitreous humor of the eye which cannot be removed, but which can easily be disregarded.

In water mounts and fresh balsam mounts one is apt to find air bubbles. To be sure that the object is an

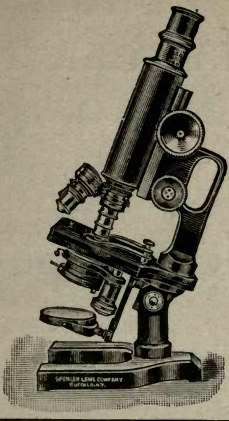
air bubble, focus up with central light. The bright spot in the center will become clearer while the edge will become darker. With oblique light the bright spot will be thrown to one side. In studying water, blood or any fluid, always cover the drop with a cover glass. The objectives are corrected for rays passing through media with parallel surfaces. If such a mount is not kept horizontal, currents will be set up, due to gravitation, and they will be seen with a magnified velocity seemingly running up hill.

The fact that the microscope reverses every movement and magnifies it may be mentioned again.

Beside any movement due to currents there is sometimes a peculiar indefinite to and fro movement of particles from one position to another. This is called Brownian movement.

In studying sections a true idea of the structure of the tissue can only be obtained by moving the slide about to bring different parts into the optical axis and by focusing with the fine adjustment to bring different levels, or optical planes, successively into view. Where serial sections are used each section must be studied in relation to its neighbors.

Sometimes sections which are freshly mounted in balsam appear cloudy and indistinct. This is because of failure to thoroughly dehydrate the specimen before putting it into the balsam. But this brings us into the realm of laboratory technique which is beyond the scope of this little volume.



*No. 20*

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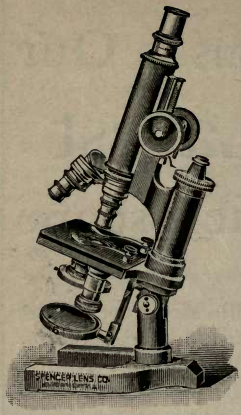


*No. 45*

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